

## EPIDEMIOLOGICAL ASPECTS OF THE BRAZILIAN SPOTTED FEVER: SEROLOGICAL SURVEY OF DOGS AND HORSES IN AN ENDEMIC AREA IN THE STATE OF SÃO PAULO, BRAZIL

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### SUMMARY

In order to obtain information on Brazilian spotted fever, a study in domestic animals was performed in the County of Pedreira, State of São Paulo, Brazil, where 17 human cases had been notified. Serum samples obtained from animals were tested by indirect immunofluorescence for detectable antibodies to spotted fever-group rickettsiae. Seropositivity was revealed in 12 (36.4%) of 33 dogs and seven (77.8%) of nine horses from the endemic area. For comparison, blood samples from dogs and horses from non endemic area were tested and four (12.9%) of 31 dogs and three (27.3%) of 11 horses were positive. The highest titers of antibodies by IFA (IgG  $\geq$  1:1024) were found only in three dogs and six horses from endemic area. The results suggest that dogs as horses may serve as environmental sentinels for establishing the prevalence of foci of spotted fever in Brazil.

**KEYWORDS:** Brazilian spotted fever; Serology; Dog; Horse.

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### INTRODUCTION

Brazilian spotted fever is the most important rickettsial disease in Brazil and its causative agent is *Rickettsia rickettsii*<sup>2-9</sup>. The natural transmission to man is through infected ticks. In Brazil, the horse tick *Amblyomma cajennense*, especially its larval and nymph stages that usually feed on people and dogs, is considered the main vector<sup>1, 15</sup>.

Human beings play no part in the propagation of the disease. Their infection is accidental and the epidemiology of this disease depends on the species of ticks responsible for the maintenance of the infection in nature<sup>11</sup>.

Considering that man as dogs and horses have common exposure to similar population of *A. cajennense*, a survey was undertaken to determine the prevalence of antibodies to *R. rickettsii* in dogs and horses in the County of Pedreira, State of São Paulo, where 17 human cases occurred since 1985.

The main objective of the present report is to demonstrate, in a Brazilian spotted fever focus, the importance not only of dogs but also of horses as indirect markers of the presence of spotted fever group rickettsiae in specific areas.

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## MATERIAL AND METHODS

### Study location

The County of Pedreira is situated at Estâncias Hidrominerais in State of São Paulo microregion. It is located in a mountainous area (latitude 22° 44' 21" S, longitude 46° 57' 27" W), 584 m above sea level. The climate is semi hot tropical with an average temperature between 30°C and 35°C. The confluence of three rural localities (Jaguari Farm, Fortaleza Farm and Workers' Colony of Nadir Figueiredo Industry), located along Jaguari River, 3.0 km from the urban center, was selected for surveillance because of the high prevalence of confirmed human cases of Brazilian spotted fever.

### Serum collection

On September/October 1994, sera were collected from two distinct apparently healthy populations of dogs ranging in estimated age from three months to 13 years and horses ranging in estimated age from 2 to 20 years. One consisted of dogs and horses residing within the endemic area. This sample represented about 90% of the resident canine population and all horses of the area. The second population, the control group, consisted of animals of other areas of the county in which no cases of spotted fever have been notified that were confined to a farm.

Blood samples were collected from the jugular vein of horses and from the brachium vein of dogs. All sera were transported to the laboratory on ice and stored at -20°C until tested.

### Serologic study

All sera were tested by indirect immunofluorescence (IFT) with antigens obtained from Vero cell culture infected by *R. rickettsii*, prepared at the Center for Disease Control, Atlanta, Georgia, USA. Fluorescein isothiocyanate-labeled rabbit anti-dog and anti-horse globulins were the test conjugate (heavy and light chain

– Sigma). Based on the study of MAGNARELLI et al.<sup>10</sup>, sera with antibodies titers  $\geq 1:64$  were considered positive.

### Statistical analysis

All data gathered were compared using the Chi-square test of significance.

## RESULTS

Twelve (36.4%) of the 33 dogs of the endemic area had positive immunofluorescent antibody titers (Table 1). The control group had only four (12.9%) of 31 dogs seropositive to rickettsiae spotted fever group. The highest titer of antibodies by IFT (IgG > 1:64) was found in dogs from endemic area.

Seven (77.8%) of nine horses of the endemic area and three (27.3%) of control 11 horses were seropositive with titer of 1:64 or greater (Table 1). Only horses from endemic area had immunofluorescent antibodies titers  $\geq 1:1024$ .

The results indicate that the prevalence of antibodies to spotted fever group-rickettsiae was significantly higher in dogs ( $p < 0.025$ ) and horses ( $p < 0.018$ ) from endemic area than in the control population.

## DISCUSSION

The epidemiology of spotted fever is intimately associated with the species of ticks and their vertebrate hosts. The incidence of the disease in humans, who are incidental victims, depends largely on the behavior of the ixodid vector, their distribution and ecology, and either on the tick's encroachment into human domain or on man's penetration of the tick's wild domain. In Brazil, *Amblyomma cajennense*, the horse tick, which also parasitizes other animals, is the primary vector of this disease.

TABLE 1

Titers of indirect immunofluorescent test (IFT) for spotted fever group rickettsia in dogs and horses of endemic (E) and non endemic (NE) areas in Pedreira, São Paulo, Brazil.

Animal	Number studied	Titers of IFT							Number of positive tests (%)
		<32	64	128	256	512	1024	2048	
E-dog	33	21	02	03	02	02	01	02	12 (30.3)*
NE-dog	31	27	04	00	00	00	00	00	04 (12.9)
E-horse	09	02	00	00	00	01	01	05	07 (77.8)**
NE-horse	11	08	00	01	01	01	00	00	03 (27.3)

\*  $X^2 = 3.4$  for dogs;  $p < 0.025$

\*\*  $X^2 = 5.0$  for horses;  $p < 0.018$

Results of serological surveys of dog populations in the eastern and southern United States, where *Derma-centor variabilis*, the American dog tick is the vector of this disease, have shown the association between seropositive dogs and human cases of Rocky Mountain spotted fever. This information provides additional evidence that dogs are not only important in facilitating exposure of people to infected ticks but a good sentinel host for human infection<sup>3-5,8,10,12,14</sup>. In Brazil, recent studies confirm the seropositivity in dogs concomitantly to human cases<sup>6,13</sup>, but there is no mention of the role of horses in the epidemiology of this rickettsiosis. In considering that the horse is the most important natural host of *A. cajennense*, it is possible that the serologic surveys of these mammals may be of particular value in indicating focal activity of spotted fever group-rickettsiae in Brazil.

Although these results do not prove that the animals have been infected with *R. rickettsii*, but only that they were infected with a member of the spotted fever group rickettsiae, the presence of seropositivity in these animals with titers  $\geq 1:1024$  associated with human confirmed cases and isolation of rickettsia from ticks in cell culture<sup>7</sup>, allow us to suggest that horses may also serve as sentinel hosts for human infection in an endemic area of Brazilian spotted fever in the County of Pedreira, State of São Paulo.

In summary, a serological evidence of infection of spotted fever in dogs and horses may contribute to the knowledge of this zoonosis in Brazil and may provide an important indicator of the presence of rickettsia in nature. This fact helps us in the definition and control of the endemic area of Brazilian spotted fever. Further investigation is needed to clarify the importance of horses in the epidemiology of human spotted fever in Brazil, their susceptibility and serologic response of infection with *R. rickettsii*.

## RESUMO

### Aspectos epidemiológicos da febre maculosa brasileira: inquérito sorológico em cães e equinos em uma área endêmica no Estado de São Paulo, Brasil

Com o objetivo de obter informações sobre a febre maculosa brasileira, um estudo em animais domésticos foi conduzido no município de Pedreira, São Paulo, Brasil, onde 17 casos humanos foram notificados. Amostras de soro obtidas de animais foram testadas pelo teste de imunofluorescência indireta para detecção de anticorpos para rickettsia do grupo da febre maculosa. Soro reatividade foi observada em 12 (36,4%) dos 33 cães e sete (77,8%) dos nove equinos procedentes da área endêmica. Para comparação, amostras de sangue de cães e de equinos procedentes de área não endêmica fo-

ram testadas e quatro (12,9%) dos 31 cães e três dos 10 equinos foram positivos. Somente três cães e seis equinos procedentes da área endêmica tinham títulos de anticorpos imunofluorescentes elevados ( $\geq 1:1024$ ). Os resultados obtidos sugerem que além dos cães, os equinos poderiam servir também como animal sentinela na febre maculosa brasileira.

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